INTERSEXUAL FEMALES AND INTERSEXUALITY IN HABROBRACON

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Despite the great amount of breeding work especially centering about a search for irregular sex types in the parasitic wasp *Habrobracon juglandis* (Ashmead), there has hitherto been reported (Whiting, Greb and Speicher, 1934) only one real intersexual form. This is the mutant type gynoid, the gene for which, gy, causes haploid males to be weakly intersexual. Gynoid females are indistinguishable from wild type. The trait acts as a recessive in heterozygous diploid males.

Gynoid males are similar to normal males in internal structure and in external genitalia. Their ocelli are large resembling those of normal males. Their normal male instincts indicate that the brain is structurally as in the male, since mating reactions in Habrobracon are determined by the brain. Sclerotization of the abdomen is progressively heavier anteriorly, approximating the condition found in the female. Antennae of normal males have about twenty segments in the flagellum, those of females usually not more than thirteen. In gynoid males the segments are reduced in number to that of the female, although they are not quite as short and thick. Superficially a gynoid male suggests a sex-mosaic or gyander with female head, male abdomen, but, as indicated, certain structures are themselves intergrading, the body is approximately symmetrical with all parts presumably of the same genetic constitution and the type is perpetuated as a pure-breeding form.

Nine intersexual females of the same species have recently been found and are herewith reported for the first time. They occurred among the offspring of a single female. Superficially these appear to be the reverse of the gynoid males, being more masculine anteriorly, feminine posteriorly. The heads are characteristically male having large ocelli and long antennae, flagellar segments ranging from 18 to 21 with 20 as the mode. Tests made on five of the nine showed indifference to caterpillars and vigorous attempts to mate with females, indicating the brain to be structurally male. Abdominal sclerotization is male-like anteriorly. The first and second tergites are thin and the anterior sternal thickenings small. Sclerotization is progressively heavier posteriorly and sternal thickenings become elongate, approximating the condition of the female.

Internal structures of the abdomen are as in the female, including normal poison sack and glands and seminal receptacle. The ovaries, however, lack differentiated nurse cells and ova. Each appears to be a pair of sacks of oögonia

similar to the primordia of the ovarioles formed in the spun-in larva and normally remaining essentially unchanged until the eyes of the pupa begin to turn black, when differentiation of oöcytes begins.

FIGURE 1.   Gynoid male.   Note the short "female" antennae and the heavy anterior sclerotization of the abdomen.   ×16.

FIGURE 2.   Intersexual female.   Note the long "male" antennae and the decrease in abdominal sclerotization anteriorly.   ×16.

Like gynoid, these intersexes differ from sex mosaics in being approximately symmetrical and similar to each other, in possessing sex intermediate characters and in occurring in a group in one fraternity as if caused by an hereditary factor rather than being scattered as single individuals.

The fraternity containing these nine intersexes was small since the mother had been discarded after eight egg-laying days,—two vials. Offspring were being classified for sex and for certain eye colors. Nothing unusual was observed in the first vial from which were obtained 13 females (+ 10, orange 3) and 11 males (+ 3, orange 8) of expected types. The nine intersexes (+ 7, orange 2) were all found in the second vial which contained 13 males (+ 4, orange 9) and no females. Proportion of wild type to orange eye color deviates from the expected 1 : 1 in the females and intersexes in the opposite direction from that
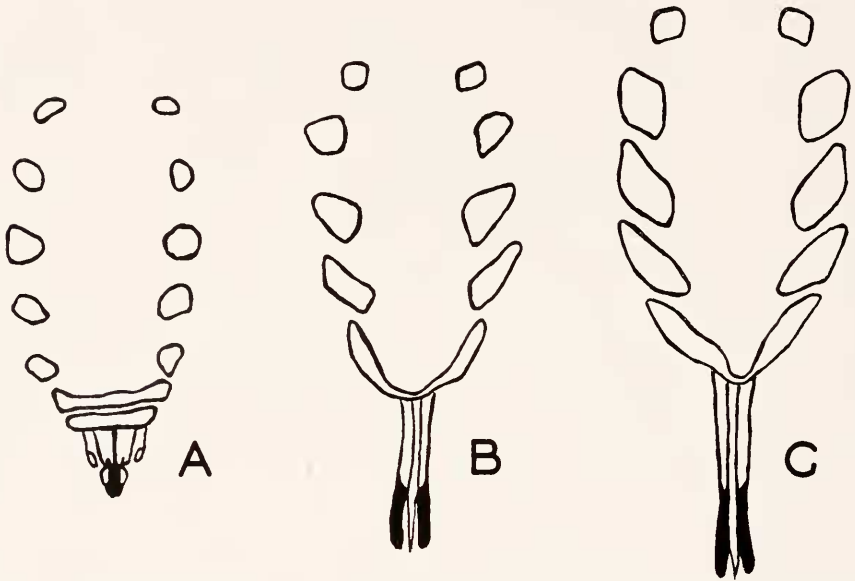

FIGURE 3.   Outlines of abdominal sternal thickenings and of external genitalia in normal male (A), intersexual female (B) and normal female (C).   ×65.

in the males. This may be but a fluctuation due to small numbers or it may indicate some chromosomal irregularity.

The males appeared structurally normal except that one had external genitalia slightly reduced, a condition not infrequently found in Habrobracon. Dissection of seven including this one showed internal genitalia normal. Flagellar segments of antennae ranged from 18 to 21 with mode 20, normal for males. Ocelli were of normal male size.

Since the offspring in the first vial had been discarded no tests could be made. A mass culture from the vial 2 males, besides individual pairings of four of these with related females, yielded nothing irregular in the immediate progeny or in later generations. Many closely related side lines, which were being bred for the eye color studies, gave only normal types. Unfortunately the eye colors were brought into the mutant fraternity in such a way as to be of no significance for determining which offspring were from fertilized, which from unfertilized eggs.

Discussion

The mother of these intersexes may have mated with two different brothers before she was isolated for breeding. Sperm from one male may have been used first to produce the females. The second male may have sired the intersexes from sperm with a dominant intersex factor. This hypothesis is regarded as unlikely in view of the fact that the females and intersexes were produced in separate vials. In known instances of double matings the two types of offspring expected eclose together, suggesting that the sperm have mixed.

If a mutation occurs in a primitive germ cell of a female of Habrobracon, the resulting mutant tissue tends to form a stratum cutting across the two ovarioles of both ovaries. This is due to the method of development from the primitive germ cell mass which separates longitudinally into the gonad primordia in a late embryonic stage. Each primordium elongates in the grown maggot, the beginning of sex-differentiation, and is subsequently divided longitudinally into two sacks, which become the ovarioles. Non-mutant tissue may then function for a period producing eggs in the first vial, for example, while heterozygous mutant tissue gives rise to eggs produced later.

If the intersexes be regarded as haploid (male) from unfertilized eggs, it may be supposed that the mother's sperm supply was exhausted before transfer to a second vial. Normal males and intersexual males would then segregate in equal ratio in vial 2. This hypothesis is regarded as unlikely because of the structure of the intersexes indicating that they are fundamentally female.

A dominant mutation in the ovaries should appear in only half of the zygotes produced while the mutant tissue is functioning. Normal females might then be expected in vial 2 at least equal in number to the intersexual. If, however, the mutation occurred in the sex-differentiating chromosome segment as a modification (deletion ?) in one of the sex factors (changing xb to xb^m), females (xa/xb) might be replaced by intersexes (xa/xb^m). Sperm, xa, fertilizing eggs from xa/xb tissue in vial 1 would produce normal females, xa/xb, and diploid males, xa/xa, but from xa/xb^m tissue in vial 2, intersexual females, xa/xb^m, and highly inviable diploid males, xa/xa, would result. Unfertilized eggs would give haploid males as expected but xb^m males might be inviable.

The data must be regarded as inadequate to prove whether these intersexes were due to a modification connected with the normally sex-differentiating factor or whether, like gynoid, to an independent change. The series of sex alleles, xa, xb, xc, etc., has been shown to be located at about the center of the left arm of the linkage map. The gene gynoid, gy, is located near the distal end of the right arm and therefore segregates independently of sex.

It is questionable whether the diverse effects of gynoid on antennae and abdominal sclerotization should be regarded as multiple effects of a single gene. Gynoid may possibly be a translocation from the differential segment determining sex, the x factor. In a male with the sex allele in the normal position this might give a complementary feminizing effect causing intersexuality.

Goldschmidt has defined an intersex as a phenotypic mosaic which begins development as one sex according to its chromosomal constitution, XY or ZZ ♂, XX or WZ ♀, and then, after a turning-point, forms organs as in the opposite sex. The earlier the turning-point, the higher the degree of intersexuality.

With sex determination as in Habrobracon, haploid intersexes should begin development as male, later shifting to female. The same should apply to diploids if homozygous for the sex factor. Diploids heterozygous for sex should begin development as female, later shifting to male.

The nine intersexual females discussed here must be regarded as more strongly intersexual than gynoid males since antennae, ocelli and instincts are completely sex reversed. The abdominal sclerotization of both intersexual types is intermediate. Neither external nor internal genitalia are affected except that the ovaries of the intersexual females fail to mature, remaining as sacks of oögonial tissue. The turning-point appears to occur earlier in the anterior than in the posterior region of the body or else the developmental processes forming the external genitalia, which are begun before the turning-point, are such that they must be carried to normal completion.

A comparison may here be made with triploid females of Habrobracon. These are daughters of diploid males arising from fertilization of a normal egg by a diploid sperm. They are presumably an unbalanced type, having two similar paternal sex alleles combined with a dissimilar member of the series of maternal origin. A diploid female may then be xa/xb, while a triploid will be xa/xa/xb or xa/xb/xb. These formulae suggest the possibility of intersexuality, but dominance relationships appear to be such that triploid females show no masculine traits either in structure or in reactions.

Their egg production is, however, considerably lowered, being about one-fourth that of diploid females. This reduction, involving both ova and nurse cells, may be a small step toward intersexuality. A compensatory growth takes place in the oögonial chamber which enlarges and elongates considerably under the influence of feeding from host caterpillars. It would be of interest to know what might have happened to the ovaries of the intersexual females if they had been similarly nourished.

The suggestion has frequently been made that diploid males may be sex-reversed females. This view originates in the older concept that diploidism as such causes femaleness, a view now shown to be erroneous. Certain differences of diploid from haploid males are to be expected dependent upon chromosome number, but these are not necessarily in the direction of femaleness. Cell-size for example is not only much greater in diploid males than in haploid: it actually surpasses considerably the cell size of the normal diploid females. The sex-linked gene "fused" causes antennal segments to be much reduced and fused together. Fused females have much shorter antennae than haploid fused males. Diploid fused males approximate fused females, having antennae only slightly longer. Wild type females have much shorter antennae than haploid wild type males. Diploid wild type males approximate haploid, but, as determined by count of segments, their antennae are slightly shorter. Difference in antennal length of diploid from haploid males is due to chromosome number as such; difference of females from diploid males is due to heterozygosis for the sex factor. As regards antennae, the gene "fused" increases the difference due to chromosome number, but tends to mask the difference due to sex. Intermediacy of diploid males with respect to antennal length should not be regarded as intersexuality.

Summary

Nine female intersexes are described and compared with the one intersexual form previously known in Habrobracon, the fertile mutant type gynoid, a weakly intersexual male.

These female intersexes proved sterile, having male heads and instincts and abortive ovaries. They are, in general, female posteriorly, while gynoid males have partially feminized heads but react like males.

Evidence suggests a dominant mutation in the sex-differentiating factor as a possible cause of this female intersexuality.

On the basis of comparison with these female intersexes, it is suggested that the structure of ovaries in triploid females represents a step toward intersexuality.

Antennal length of diploid males, both fused and wild type, although intergrading, is not regarded as due to intersexuality but to diploidy as such.

LITERATURE CITED

Whiting, P. W., Raymond J. Greb and B. R. Speicher, 1934. A new type of sex-intergrade. *Biol. Bull.*, **66**: 152–165.